



Schirò, G., Brogan, A. P. S., & et al. (2021). Diffusivelike Motions in a Solvent-Free Protein-Polymer Hybrid. *Physical Review Letters*, 126(8), [088102]. <https://doi.org/10.1103/PhysRevLett.126.088102>

Publisher's PDF, also known as Version of record

Link to published version (if available):
[10.1103/PhysRevLett.126.088102](https://doi.org/10.1103/PhysRevLett.126.088102)

[Link to publication record in Explore Bristol Research](#)
PDF-document

This is the final published version of the article (version of record). It first appeared online via American Physical Society at <https://doi.org/10.1103/PhysRevLett.126.088102> . Please refer to any applicable terms of use of the publisher.

University of Bristol - Explore Bristol Research

General rights

This document is made available in accordance with publisher policies. Please cite only the published version using the reference above. Full terms of use are available:
<http://www.bristol.ac.uk/red/research-policy/pure/user-guides/ebr-terms/>

Diffusivelike Motions in a Solvent-Free Protein-Polymer Hybrid

Giorgio Schirò^{1,*†}, Yann Fichou^{2,*‡}, Alex P. S. Brogan³, Richard Sessions⁴, Wiebke Lohstroh⁵,
 Michaela Zamponi⁶, Gerald J. Schneider⁷, François-Xavier Gallat¹, Alessandro Paciaroni⁸,
 Douglas J. Tobias⁹, Adam Perriman¹⁰, and Martin Weik^{1,§}

¹*Université Grenoble Alpes, CEA, CNRS, Institut de Biologie Structurale, F-38000 Grenoble, France*

²*CNRS, Chemistry and Biology of Membranes and Nanoobjects (CBMN) UMR 5348, Institut Européen de Chimie et Biologie (IECB),
 University of Bordeaux, 33600 Pessac, France*

³*School of Biochemistry, Medical Sciences Building, University Walk, Bristol BS8 1TD, United Kingdom*

⁴*Department of Chemistry, King's College London, Britannia House, London SE1 1DB, United Kingdom*

⁵*Heinz Maier-Leibnitz Zentrum, Technische Universität München, Garching, Germany*

⁶*Forschungszentrum Jülich GmbH, Jülich Centre for Neutron Science at Heinz Maier-Leibnitz Zentrum, Garching, Germany*

⁷*Department of Chemistry and Department of Physics & Astronomy, Louisiana State University, Baton Rouge, Louisiana 70803, USA*

⁸*Dipartimento di Fisica e Geologia, Università degli Studi di Perugia, 06123 Perugia, Italy*

⁹*Department of Chemistry, University of California, Irvine, California, CA 92697, USA*

¹⁰*School of Cellular and Molecular Medicine, University of Bristol, Bristol BS8 1TL, United Kingdom*



(Received 4 July 2020; accepted 11 January 2021; published 25 February 2021)

The interaction between proteins and hydration water stabilizes protein structure and promotes functional dynamics, with water translational motions enabling protein flexibility. Engineered solvent-free protein-polymer hybrids have been shown to preserve protein structure, function, and dynamics. Here, we used neutron scattering, protein and polymer perdeuteration, and molecular dynamics simulations to explore how a polymer dynamically replaces water. Even though relaxation rates and vibrational properties are strongly modified in polymer coated compared to hydrated proteins, liquidlike polymer dynamics appear to plasticize the conjugated protein in a qualitatively similar way as do hydration-water translational motions.

DOI: [10.1103/PhysRevLett.126.088102](https://doi.org/10.1103/PhysRevLett.126.088102)

The coupling to hydration water via a dynamic hydrogen bond network endows proteins with the internal dynamics required for being biologically active [1]. Removing hydration water from the surface of a protein is known to generally suppress biological activity by inhibiting macromolecular conformational flexibility [2]. Neutron scattering is a technique largely used to probe protein dynamics on the picosecond-nanosecond time-scale [3] by, e.g., measuring atomic mean square displacements (MSD). The temperature dependence of MSDs in a hydrated protein reveals the onset of protein flexibility at about 240 K (the so-called protein dynamical transition [4]), which is suppressed upon water removal [5]. The onset of water center-of-mass translational motions has been proposed as the atomistic mechanism able to plasticize proteins through the hydrogen bond (HB) interaction of water with their surface. The finding was first predicted by molecular dynamics (MD) simulations [6,7] and later verified and complemented by quasielastic neutron scattering (QENS) experiments [8]. This physical scenario was challenged when solvent-free protein-polymer surfactant nanohybrids were engineered [9–14]. Indeed, a myoglobin-polymer hybrid was shown to be structurally intact [11], hyperthermophilic [11], biologically active [9], and still displaying a dynamical

transition at about 250 K analogous to that observed in hydrated myoglobin (Mb) [15].

However, the determination of MSDs [15,16] provides only limited information on the nature of the underlying dynamics. In particular, the dynamical transition can be interpreted either as a thermal population of a temperature independent number of degrees of freedom or as a temperature dependent structural evolution activating additional degrees of freedom. A detailed description of the (thermo) dynamical properties that emulate hydration mechanisms in solvent-free protein-polymer hybrids remains elusive. Recent efforts in the field of protein-polymer nanohybrids are devoted to fine-tuning the activity of the embedded protein by adjusting the polymer layer properties [17]. Neutron scattering has been used already to study the influence of the length [18] and the number [16,19] of polymer chains on the structure and dynamics of the hybrids. Here, we used QENS and inelastic neutron scattering (INS) in combination with MD simulations to unravel the physical basis for the activity and dynamics of a Mb-polymer hybrid and to show the similarities and differences between the dynamics of protein-polymer hybrids and hydrated proteins.

The Mb-polymer hybrid was synthesized by electrostatically grafting anionic surfactant chains (glycolic acid

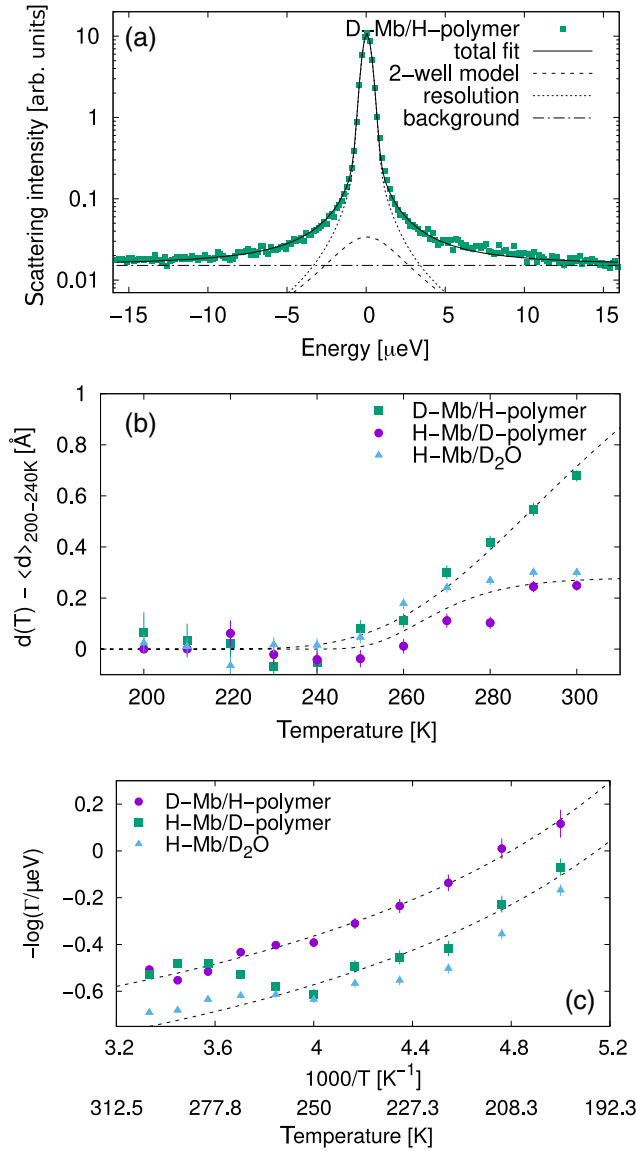


FIG. 1. (a) example of fitting results for the spectrum of D-Mb/H-polymer at 260 K and $q = 0.78 \text{ \AA}^{-1}$. (b) jump distance d (see inset and SM for details) as a function of temperature upon subtraction of the average in the 200–240 K temperature range. Dashed lines are to guide the eye. (c) Arrhenius plot of parameter Γ . Dashed lines are fitting curves with a VFT function in the whole temperature range (D-Mb/H-polymer) and up to 250 K (H-Mb/D-polymer).

ethoxylate lauryl ether) to the surface of Mb cationized with *N*, *N*-dimethyl-1,3-propanediamine [9,15]. Details on the preparation of the samples used here have been published elsewhere [9,15]. Two different Mb-polymer hybrids had been prepared by selective perdeuteration: hydrogenated Mb conjugated with deuterated polymer (H-Mb/D-polymer) and deuterated Mb conjugated with hydrogenated polymer (D-Mb/H-polymer). Because of the dominating incoherent signal from hydrogen nuclei, about 89% (91%) of the incoherent scattering signal originates from

Mb (polymer) dynamics in the H-Mb/D-polymer (D-Mb/H-polymer) sample [15]. A fully hydrogenated sample had also been prepared (H-Mb/H-polymer). A fourth sample consisted in a freeze-dried powder of hydrogenated Mb hydrated with D_2O at a level of $0.43 \text{ g } D_2O/\text{g Mb}$ (H-Mb/ D_2O). In the H-Mb/ D_2O sample, 98% of the incoherent scattering signal originates from protein hydrogen atoms. The coherent signal accounts for less than 20% of the total scattering signal in all the samples investigated [15].

QENS experiments, which probe motions on the picosecond-to-nanosecond timescale, were carried out on H-Mb/D-polymer, D-Mb/H-polymer, and H-Mb/ D_2O between 200 and 300 K, on the SPHERES [20,21] spectrometer (energy resolution $\sim 0.7 \text{ \mu eV}$ FWHM) operated by JCNs at the Heinz Maier-Leibnitz Zentrum (MLZ), Garching, Germany. QENS spectra of both H-Mb/D-polymer and D-Mb/H-polymer can be approximated in the explored momentum transfer range ($0.6 < q < 1.8 \text{ \AA}^{-1}$) by a single Lorentzian term with a width independent of q , as expected, if we consider that the dynamics of hydrogen atoms bound to the polymer or to Mb are intrinsically confined. To obtain a quantitative description from the experimental data, we used a two-well model where the hydrogen motion is modeled as a jump between two different sites separated by a jump distance d , corresponding to a potential composed of two energy minima with energy difference ΔG and energy barrier ΔG^* [inset of Fig. 1(b)] [4,22]. The scattering function $S(q, \omega)$ corresponding to the two-well model can be derived with a semiclassical treatment [23] and applied to the analysis of neutron scattering data, as described in the literature [22,24] and summarized in the Supplemental Material (SM) [25].

In Fig. 1(a), an example of the fitting results is shown for D-Mb/H polymer at 260 K and at $q = 0.78 \text{ \AA}^{-1}$. Similar results are obtained at all other temperatures and q values (Fig. S1 in the SM). Figure 1(b) shows the change in the jump distance d relative to the low-temperature value (~ 3.6 and $\sim 3.7 \text{ \AA}$ for H-Mb/D-polymer and D-Mb/H-polymer, respectively, in agreement with previous data [22]) as a function of temperature. Interestingly, there is a clear difference between the protein (H-Mb/D-polymer) and the polymer (D-Mb/H-polymer) dynamics: d is barely dependent on temperature in the protein, while it clearly increases with temperature above 250 K in the polymer. Mb embedded either in the polymer matrix or in hydration water are characterized by a similar temperature dependence of d [Fig. 1(b)], indicating that motions accessed by the proteins in the two different environments at temperatures above the dynamical transition are of the same nature, even if the MSDs measured by neutron scattering are larger in the hydrated protein [15]. In Fig. 1(c), we report the temperature dependence of the rate Γ of the hopping process between the two minima [22]. Γ follows a

super-Arrhenius temperature dependence that can be approximated by a Vogel-Fulcher-Tamman (VFT) function $\Gamma = \Gamma_0 e^{-DT^*/(T-T^*)}$ in the entire temperature range for D-Mb/H-polymer, but only at low temperature for H-Mb/D-polymer. The two curves evolve in parallel below 250 K and above 270 K [Fig. 1(c)] (i.e., they have the same fragility parameter $D \sim 0.5$ and very similar divergence temperature $T^* \sim 140$ K), suggesting that the two systems are dynamically coupled [28]. Preexponential factors Γ_0 are different below 250 K and similar above 270 K, implying that polymer and protein motions occur on a different timescale at low temperature and on the same timescale above the dynamical transition. Above ~ 250 K the relaxation rate Γ of H-Mb/D-polymer approaches that of D-Mb/H-polymer. The parameter Γ of H-Mb/D₂O is also shown in Fig. 1(c). Its temperature dependence is consistent with that of H-Mb/D-polymer up to ~ 250 K, while at temperatures above ~ 250 K the relaxation rate is systematically higher in H-Mb/D₂O, in agreement with the fact that the MSDs measured by neutron scattering are larger in the hydrated protein [15].

Thermodynamic transitions in the protein-polymer hybrid were investigated by differential scanning calorimetry (DSC). A glass transition is detected in H-Mb/H-polymer with an onset temperature $T_g \sim 200$ K (see Fig. S2 in the SM), compatible with the divergence temperature $T^* \sim 140$ K of the VFT function, which is usually lower than T_g [29]. Analogous DSC data on hydrated Mb have shown that hydration water undergoes a glass transition and a first-order-like endothermic transition with onset temperatures at ~ 180 K and ~ 240 K, respectively [30].

In order to characterize the nature of motions with atomic details, we employed MD simulations that probe the same nanosecond-to-picosecond timescale as QENS. The Mb-polymer hybrid was previously modeled and shown to accurately reflect the structural properties of the Mb-polymer hybrid sample [31]. Here, we have used this model to simulate atomic trajectories at different temperatures, between 200 and 300 K, and assessed its validity to accurately reproduce the sample dynamics by computing the scattering function $S(q, \omega)$ from atomic trajectories and comparing them with the QENS data (Figs. S3 and S4 in the SM). The agreement of simulated and experimental data in the 200 to 260 K range is remarkable for both proteins (Fig. S3 in the SM) and polymer (Fig. S4 in the SM). At 280 and 300 K, the experimental QENS signals are higher than the corresponding computed values, revealing that the simulations underestimate the dynamics. This underestimation might originate from the limited accuracy of the forcefield or modeling of the protein-polymer hybrid. We first report the hydrogen displacement probability densities, which represent the distance between an atom at $t = 0$ ps and the same atom at $t = x$ ps ($x = 100$ ps, 1000 ps, and 5000 ps) in Fig. 2 at 260 K. Protein hydrogens show two distinct peaks at around 0.5 and 1.8 Å,

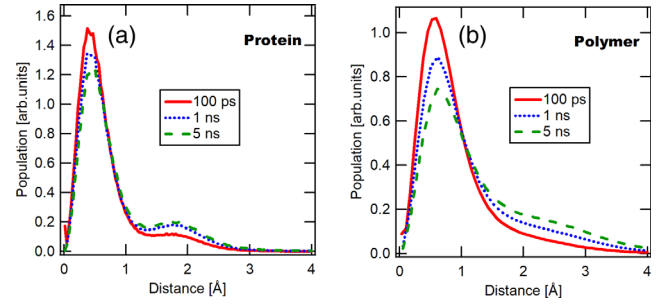


FIG. 2. Displacement probability densities of protein hydrogens (a) and polymer hydrogens (b) at 260 K after 100 ps (red), 1 ns (blue), and 5 ns (magenta).

the position of which does not change with time, recalling confined motions [Fig. 2(a)]. The peak at 1.8 Å originates from methyl-hydrogen rotations (see Fig. S5 in the SM), and the one at 0.5 Å from all other atoms. Conversely, the polymer hydrogens show a significant population for which displacement probability does not exhibit a characteristic distance, but rather a tail (above 1.5 Å), which extends to longer distances with increasing displacement times [Fig. 2(b)]. This tail reflects diffusivelike dynamics, where atoms spread away from their initial position. A similar difference between protein and polymer motions is observed at different temperatures between 200 and 300 K (Fig. S6 in the SM).

We next compared the simulation of the polymer with that of hydration water. As the reference of a hydrated protein, we simulated the powder of the maltose binding protein (MBP) hydrated at 0.4 g H₂O/g protein, which has been validated with experimental data in previous work [8]. Noting that similar hydration properties were found for the MBP and for the intrinsically disordered protein tau [8], we assume that MBP and Mb hydration resemble each other even more closely. The hydrogen displacement probability densities [Fig. 3(a)] indicate the same diffusivelike dynamics on the 100 ps timescale in hydration water and in the polymer corona [Fig. 2(b)]. Yet, the one of water hydrogens

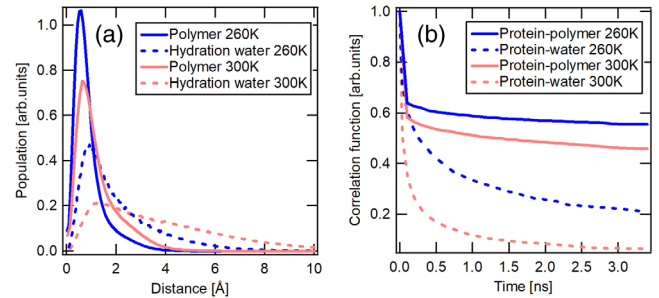


FIG. 3. (a) Displacement probability densities of polymer hydrogens (continuous lines) and hydration water (dashed lines) at 260 K (blue) and 300 K (red) after 100 ps. (b) polymer-protein (continuous lines) and water-protein (dashed lines) HB correlation function at 260 K (blue) and 300 K (red).

extends to larger distances, most likely originating from larger diffusion coefficients of water molecules. Note that when probed on the fs time scale, hydrogen dynamics of protein hydration water results in more localized dynamics [32]. Figure 3(b) represents the hydrogen bond (HB) correlation function [6], reporting on the dynamics of the HB network, for MBP water (data extracted from [8]) and Mb polymer. Mb-polymer HBs are significantly slower to break than typical protein-water HBs (Fig. 3(b)). The HB relaxation time (defined as the time at which the correlation function has decayed to $1/e = 0.368$) is 226 times shorter at 300 K for the MBP-water system (91 ps) than for the Mb-polymer system (21 ns), confirming a faster dynamics for hydration water compared to the polymer corona.

The low-frequency vibrational spectra of Mb either embedded in the polymer corona (H-Mb/D-polymer) or in hydration water (H-Mb/D₂O) were measured by INS (TOFTOF spectrometer, MLZ) and compared. In Fig. 4 we show the inelastic spectra collected at 140 K and at $q = 1.8 \text{ \AA}^{-1}$. At this temperature the modes responsible for the so-called boson peak are already sufficiently populated to be detected on the energy gain side of the INS spectrum and, at the same time, the QENS contribution is still negligible [33]. The Boson peak is visible at 2 meV and at 3.5 meV for the H-Mb/D-polymer and the H-Mb/D₂O sample, respectively (Fig. 4), indicating that vibrational modes are softer in the former than the latter. A similar softening has been observed in dry compared to hydrated protein powders [34] and was attributed to the absence of the water dissipative bath for low-frequency vibrations in the dry proteins. The softening observed in the H-Mb/D-polymer sample indicates that the polymer matrix is not able to mimic the water dissipative effect. The vibrational modes of the polymer matrix (D-Mb/H-polymer sample) are peaked at about 1.6 meV and are thus even softer than those of the conjugated protein (see Fig. 4). In Fig. 4 these modes are compared with the modes observed in the hydration water of perdeuterated MBP [33] (data reported as D-MBP/H₂O in Fig. 4) and of hydrogenated Mb (data extracted from the literature [35]).

A first conclusion from our QENS analysis is that the nature of polymer and protein dynamics is qualitatively similar, as the same physical model can be used to fit the data from both H-Mb/D-polymer and D-Mb/H-polymer [Fig. 1(a) and Fig. S1 in the SM]. The parameters obtained from fitting, however, reveal significant differences. At temperatures above ~ 250 K the jump distance d has a steplike temperature dependence in Mb, while it markedly increases in the polymer [Fig. 1(b)], revealing diffusivelike motions in the polymer that are absent in the protein. This contrast between confined motions in the protein and diffusivelike motions in the polymer was confirmed by MD simulations (Fig. 2). The increase in d occurring above ~ 250 K [Fig. 1(b)] indicates a dynamical change in the

polymer. In energetic terms, the states accessible to the polymer at temperatures above 250 K correspond to structural configurations where hydrogen atoms perform larger-amplitude motions. Conversely, the interactions stabilizing protein conformations do not allow a change in the conformational degrees of freedom of the protein. The change in the polymer structural dynamics revealed by the temperature dependence of d [Fig. 1(b)] correlates with a change in the temperature dependence of Mb energetic parameters. The temperature dependence of the free energy difference ΔG has an inflection point at ~ 250 K (Fig. S8 in the SM) from $\Delta G \sim 50$ kJ/mol at low temperature to $\Delta G \sim 10$ kJ/mol at high temperature, revealing that higher energy states become thermally accessible to the protein above ~ 250 K. The possibility for protein hydrogen atoms to visit these states gives rise to the large amplitude motions revealed by the steep increase in MSDs at temperatures above the dynamical transition [15]. The temperature dependence of the rate Γ shows a clear change in the same temperature region and becomes similar for the polymer and the protein above ~ 250 K [Fig. 1(c)], revealing a strong coupling between them.

The dynamical behavior of the protein-polymer hybrid can be summarized as follows. The polymer corona undergoes a structural-dynamical change related to a calorimetric glass transition at ~ 200 K (Fig. S2 in the SM), which is detected at 250 K on the timescale probed by the neutron spectrometer (nanosecond-picosecond). Above this transition, the polymer conformational freedom is progressively enhanced via an increase of the atomic confinement volume, mimicking diffusivelike motions. The time and space scales accessible by the QENS data presented here allow to probe the local segmental motion of polymers [36] activated above the glass transition. Structural evidence from small angle scattering and simulations [31] and our QENS data suggest a geometrical confinement of polymer chains on the nanosecond-picosecond timescale, yet our data do not enable us to discern if such a confinement gives rise to a reptation motion [37,38]. The dynamics of the protein, which is embedded in the polymer corona and interacts electrostatically with it, correlates with the change in polymer dynamics described above: at ~ 250 K, higher energy states become accessible and the rate of anharmonic motions starts following exactly that of the polymer, originating in the dynamical transition evidenced by MSDs [15]. We note that other protein-polymer hybrids investigated recently do not show the same behavior: they lack a dynamical transition in the absence of water, as well as the polymer glass transition [39].

The dynamical properties of the polymer qualitatively resemble those of hydration water previously analyzed by QENS and MD simulations [8]. Indeed, the polymer (i) shows diffusivelike motions (Fig. 2), (ii) exhibits an apparent dynamical and thermodynamical transition at ~ 240 K [Fig. 1(b)], and (iii) is dynamically coupled to

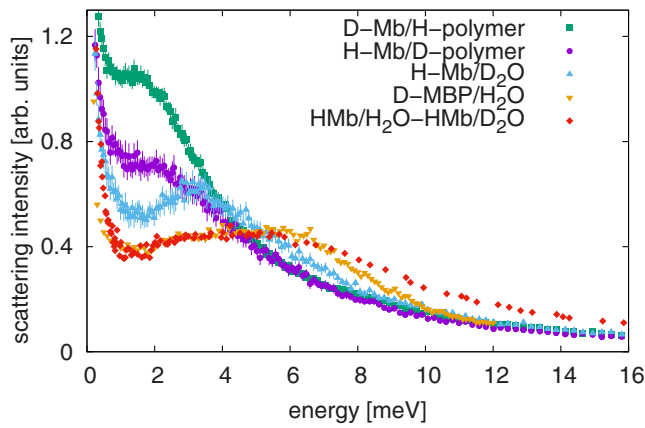


FIG. 4. Inelastic spectra of D-Mb/H-polymer (green squares), H-Mb/D-polymer (pink circles), H-Mb/D₂O (cyan triangles), and D-MBP/H₂O (orange triangles) at 140 K and $q = 1.8 \text{ \AA}^{-1}$. The spectrum of H-Mb/H₂O-H-Mb/D₂O, collected at 180 K at $q = 2 \text{ \AA}^{-1}$ [35], was scaled to 140 K by the Bose occupation factor $n(E, T) = 1/(e^{E/kT} - 1)$, where E is the energy and k the Boltzmann constant.

the protein, in particular above this temperature [Fig. 1(c)]. However, there are quantitative differences in the amplitude of the dynamics between the polymer and hydration water, as shown by MD simulations. Diffusivelike motions observed in both polymer and water are more populated and have a higher diffusion coefficient in hydration water [Fig. 3(a)]. In addition, the protein-polymer HB network is significantly less dynamic than the protein-water HB network [Fig. 3(b)]. The lower dynamics of the polymer compared to hydration water corroborates the previous proposal that the reduced conformational freedom of the polymer might allow an increased stabilization of the protein in the hybrid [11]. Although the mb-polymer hybrid has been shown to be biologically active, the slower dynamics in the polymer compared to hydration water is likely to affect the dynamics of the protein activity, in agreement with the previous observation that O₂ binds much faster to hydrated Mb than to Mb in the hybrid [9].

INS data indicate that Mb vibrational properties are strongly affected by the polymer corona. Mb low-frequency modes are softened in the hybrid with respect to the hydrated protein, most likely due to both the different nature of protein interactions with its environment (polymer or water) and the distribution of vibrational modes of the polymer matrix. The importance of protein low-frequency modes in determining the dynamics accompanying functional reactions at catalytic sites has been demonstrated [40,41] and recently reviewed [42]. We speculate that replacing hydration water by a polymer corona, even if the overall Mb flexibility is preserved [9,15], affects protein reactivity by suppressing vibrational modes directly involved in functional protein processes like ligand binding and release [41,43]. Further developments of protein-polymer hybrids for specific biotechnological applications

might focus on conserving the vibrational properties of hydrated proteins and increasing the polymer dynamics. This might be achieved, for instance, by weakening the interpolymer interactions or by lowering the molecular weight of the polymer to reduce the microscopic viscosity.

We are indebted to Laurent Heux for the use of calorimeter at CERMAV (Grenoble) and Pierre Sailer for the assistance during the calorimetry experiment. We thank Matthias Heyden for providing his custom-made trajectory analysis software. Financial support by the CEA, the CNRS, and the U. G. A. is acknowledged, as well as a grant from the Agence Nationale de la Recherche (Project No. ANR-11-BSV5-027) to M. W. The I. B. S. acknowledges integration into the Interdisciplinary Research Institute of Grenoble (IRIG, CEA). This work used the platforms of the Grenoble Instruct-ERIC center (ISBG ; UMS 3518 CNRS-CEA-UGA-EMBL) within the Grenoble Partnership for Structural Biology (PSB), supported by FRISBI (ANR-10-INBS-0005-02) and GRAL, financed within the University Grenoble Alpes graduate school (Ecoles Universitaires de Recherche) CBH-EUR-GS (ANR-17-EURE-0003). This project has received funding from the European Union's 7th Framework Programme for research, technological development and demonstration under the NMI3-II Grant No. 283883. We thank the UKRI for support for AWP (MR/S016430/1 and EP/K026720/1).

*These authors contributed equally to the work.

[†]giorgio.schiro@ibs.fr

[‡]y.fichou@iecb.u-bordeaux.fr

[§]martin.weik@ibs.fr

- [1] G. Schirò and M. Weik, Role of hydration water in the onset of protein structural dynamics, *J. Phys. Condens. Matter* **31**, 463002 (2019).
- [2] J. A. Rupley and G. Careri, Protein hydration and function, *Adv. Protein Chem.* **41**, 37 (1991).
- [3] F. Gabel, D. Bicout, U. Lehnert, M. Tehei, M. Weik, and G. Zaccari, Protein dynamics studied by neutron scattering, *Q. Rev. Biophys.* **35**, 327 (2002).
- [4] W. Doster, S. Cusack, and W. Petry, Dynamical transition of myoglobin revealed by inelastic neutron scattering, *Nature (London)* **337**, 754 (1989).
- [5] T. Kleinert, W. Doster, F. Post, and M. Settles, Hydration effects on protein function: The kinetics of ligand binding to myoglobin, in *Proceedings of the Italian Physical Society*, edited by M. U. Palma, M. B. Palma-Vittorelli, and F. Parak (1992), Vol. 43, pp. 127–130.
- [6] M. Tarek and D. J. Tobias, Role of Protein-Water Hydrogen Bond Dynamics in the Protein Dynamical Transition, *Phys. Rev. Lett.* **88**, 138101 (2002).
- [7] A. L. Tournier, J. Xu, and J. C. Smith, Translational hydration water dynamics drives the protein glass transition, *Biophys. J.* **85**, 1871 (2003).
- [8] G. Schirò, Y. Fichou, F.-X. Gallat, K. Wood, F. Gabel, M. Moulin, M. Härtlein, M. Heyden, J.-P. Colletier, A. Orecchini, A. Paciaroni, J. Wuttke, D. J. Tobias, and

- M. Weik, Translational diffusion of hydration water correlates with functional motions in folded and intrinsically disordered proteins, *Nat. Commun.* **6**, 6490 (2015).
- [9] A. W. Perriman, A. P. S. Brogan, H. Cölfen, N. Tsoureas, G. R. Owen, and S. Mann, Reversible dioxygen binding in solvent-free liquid myoglobin, *Nat. Chem.* **2**, 622 (2010).
- [10] A. W. Perriman and S. Mann, Liquid proteins—a new frontier for biomolecule-based nanoscience, *ACS Nano* **5**, 6085 (2011).
- [11] A. P. S. Brogan, G. Siligardi, R. Hussain, A. W. Perriman, and S. Mann, Hyper-thermal stability and unprecedented re-folding of solvent-free liquid myoglobin, *Chem. Sci.* **3**, 1839 (2012).
- [12] A. P. S. Brogan, K. P. Sharma, A. W. Perriman, and S. Mann, Enzyme activity in liquid lipase melts as a step towards solvent-free biology at 150 °C, *Nat. Commun.* **5**, 5058 (2014).
- [13] A. P. S. Brogan, K. P. Sharma, A. W. Perriman, and S. Mann, Isolation of a highly reactive β -sheet-rich intermediate of lysozyme in a solvent-free liquid phase, *J. Phys. Chem. B* **117**, 8400 (2013).
- [14] K. P. Sharma, K. Bradley, A. P. S. Brogan, S. Mann, A. W. Perriman, and D. J. Fermin, Redox transitions in an electrolyte-free myoglobin fluid, *J. Am. Chem. Soc.* **135**, 18311 (2013).
- [15] F.-X. Gallat, A. P. S. Brogan, Y. Fichou, N. McGrath, M. Moulin, M. Härtlein, J. Combet, J. Wuttke, S. Mann, G. Zaccai, C. J. Jackson, A. W. Perriman, and M. Weik, A polymer surfactant corona dynamically replaces water in solvent-free protein liquids and ensures macromolecular flexibility and activity, *J. Am. Chem. Soc.* **134**, 13168 (2012).
- [16] D. Russo, A. De Angelis, A. Paciaroni, B. Frick, N. de Sousa, F. R. Wurm, and J. Teixeira, Protein-polymer dynamics as affected by polymer coating and interactions, *Langmuir* **35**, 2674 (2019).
- [17] R. Chapman and M. H. Stenzel, All wrapped up: Stabilization of enzymes within single enzyme nanoparticles, *J. Am. Chem. Soc.* **141**, 2754 (2019).
- [18] C. Le Cœur, S. Combet, G. Carrot, P. Busch, J. Teixeira, and S. Longeville, Conformation of the poly(ethylene glycol) chains in DiPEGylated hemoglobin specifically probed by SANS: Correlation with PEG length and in vivo efficiency, *Langmuir* **31**, 8402 (2015).
- [19] D. Russo, C. Pelosi, F. R. Wurm, B. Frick, J. Ollivier, and J. Teixeira, Insight into protein-polymer conjugate relaxation dynamics: The importance of polymer grafting, *Macromol. Biosci.* **20**, 1900410 (2020).
- [20] J. Wuttke, A. Budwig, M. Drochner, H. Kämmerling, F.-J. Kayser, H. Kleines, V. Ossóvyi, L. C. Pardo, M. Prager, D. Richter, G. J. Schneider, H. Schneider, and S. Stanger, SPHERES, Jülich's high-flux neutron backscattering spectrometer at FRM II. *Rev. Sci. Instrum.* **83**, 075109 (2012).
- [21] M. Zamponi and M. Khanef, SPHERES: Backscattering spectrometer, *J. Large-Scale Res. Facil.* **1**, A30 (2015).
- [22] G. Schiró, F. Natali, and A. Cupane, Physical Origin of Anharmonic Dynamics in Proteins: New Insights from Resolution-Dependent Neutron Scattering on Homomeric Polypeptides, *Phys. Rev. Lett.* **109**, 128102 (2012).
- [23] A. Stoeckli, A. Furrer, C. H. Schoenenberger, B. H. Meier, R. R. Ernst, and I. Anderson, Dynamics of hydrogen bonds in carboxylic acids, *Physica (Amsterdam)* **136B+C**, 161 (1986).
- [24] M. Bée, *Quasielastic Neutron Scattering* (Adam Hilger, Bristol, 1988).
- [25] See Supplemental Material at <http://link.aps.org/supplemental/10.1103/PhysRevLett.126.088102> for details on theoretical models and data analysis and for supplemental data, which includes Refs. [26,27].
- [26] M. Bée, *Quasielastic Neutron Scattering* (Adam Hilger, Bristol, 1988).
- [27] A. Luzar and D. Chandler, Effect of Environment on Hydrogen Bond Dynamics in Liquid Water, *Phys. Rev. Lett.* **76**, 928 (1996).
- [28] P. W. Fenimore, H. Frauenfelder, B. H. McMahon, and F. G. Parak, Slaving: Solvent fluctuations dominate protein dynamics and functions, *Proc. Natl. Acad. Sci. U. S. A.* **99**, 16047 (2002).
- [29] N. Metatla and A. Soldera, The Vogel-Fulcher-Tamman equation investigated by atomistic simulation with regard to the Adam-Gibbs model, *Macromolecules* **40**, 9680 (2007).
- [30] G. Schiró, M. Fomina, and A. Cupane, Communication: Protein dynamical transition vs. liquid-liquid phase transition in protein hydration water, *J. Chem. Phys.* **139**, 121102 (2013).
- [31] A. P. S. Brogan, R. B. Sessions, A. W. Perriman, and S. Mann, Molecular dynamics simulations reveal a dielectric-responsive coronal structure in protein-polymer surfactant hybrid nanoconstructs, *J. Am. Chem. Soc.* **136**, 16824 (2014).
- [32] S. E. Pagnotta, F. Bruni, R. Senesi, and A. Pietropaolo, Quantum behavior of water protons in protein hydration shell, *Biophys. J.* **96**, 1939 (2009).
- [33] A. Paciaroni, A. Orecchini, E. Cornicchi, M. Marconi, C. Petrillo, M. Härtlein, M. Moulin, H. Schober, M. Tarek, and F. Sacchetti, Fingerprints of Amorphous Ice-like Behavior in the Vibrational Density of States of Protein Hydration Water, *Phys. Rev. Lett.* **101**, 148104 (2008).
- [34] H. Leyser, W. Doster, and M. Diehl, Far-Infrared Emission by Boson Peak Vibrations in a Globular Protein, *Phys. Rev. Lett.* **82**, 2987 (1999).
- [35] M. Settles and W. Doster, Anomalous diffusion of adsorbed water: A neutron scattering study of hydrated myoglobin, *Faraday Discuss.* **103**, 269 (1996).
- [36] J. Colmenero and A. Arbe, Recent progress on polymer dynamics by neutron scattering: From simple polymers to complex materials, *J. Polym. Sci. B* **51**, 87 (2013).
- [37] P. G. de Gennes, Reptation of a polymer chain in the presence of fixed obstacles, *J. Chem. Phys.* **55**, 572 (1971).
- [38] P. Schlegel, B. Farago, C. Lartigue, A. Kollmar, and D. Richter, Clear Evidence of Reptation in Polyethylene from Neutron Spin-Echo Spectroscopy, *Phys. Rev. Lett.* **81**, 124 (1998).
- [39] D. Russo, M. Plazanet, J. Teixeira, M. Moulin, M. Härtlein, F. R. Wurm, and T. Steinbach, Investigation into the relaxation dynamics of polymer-protein conjugates reveals surprising role of polymer solvation on inherent protein flexibility, *Biomacromolecules* **17**, 141 (2016).

- [40] G. Li, D. Magana, and R. B. Dyer, Anisotropic energy flow and allosteric ligand binding in albumin, *Nat. Commun.* **5**, 3100 (2014).
- [41] M. Levantino, G. Schirò, H. T. Lemke, G. Cottone, J. M. Glowia, D. Zhu, M. Chollet, H. Ihee, A. Cupane, and M. Cammarata, Ultrafast myoglobin structural dynamics observed with an x-ray free-electron laser, *Nat. Commun.* **6**, 6772 (2015).
- [42] C. M. Cheatum, Low-frequency protein motions coupled to catalytic sites, *Annu. Rev. Phys. Chem.* **71**, 267 (2020).
- [43] K. A. Niessen, M. Xu, D. K. George, M. C. Chen, A. R. Ferré-D'Amaré, E. H. Snell, V. Cody, J. Pace, M. Schmidt, and A. G. Markelz, Protein and RNA dynamical fingerprinting, *Nat. Commun.* **10**, 1026 (2019).